The Animal Pigment Bilirubin Discovered in Plants

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Supporting Information

Isolation of Bilirubin from Plant Aril

Plant arils were removed from the seed and extracted with chloroform until the extracts were colorless. Aril lipids were isolated by re-dissolving the evaporated extract in DMSO (3 mL per gram of extract) and extracting with hexane in a separatory funnel. The hexane layer was evaporated and the lipid residue stored for future study. The DMSO layer, which contained the pigment, was purified further by preparative HPLC using an XTerra MS $\mathbf{C_{18}}$ OBD column (5 µm, 50mm, 19mm i.d., Waters), and the binary solvent system developed by Spivak and Yuey[3]. The flow rate was 3 mL per minute. The bilirubin fraction, which eluted from 9 to 13 minutes, was collected from a series of runs, combined, diluted with HPLC grade water (1 mL eluate to 2.5 mL water), and passed through a Waters Sep-Pak C-18 cartridge. Bound pigments were washed with water to remove salts, then acetone, which was a co-solvent for water, and eluted with chloroform until eluates were colorless. The chloroform was evaporated under reduced pressure. Pigments were dried in a vacuum desiccator and stored at -80C. Analytical HPLC analyses were performed on a reversed phase ODS-A column (5µm, 150mm * 4.3 mm i.d., Waters). The solvents and gradient were the same as those used for the preparative HPLC work. The flow rate was 1 mL per minute.

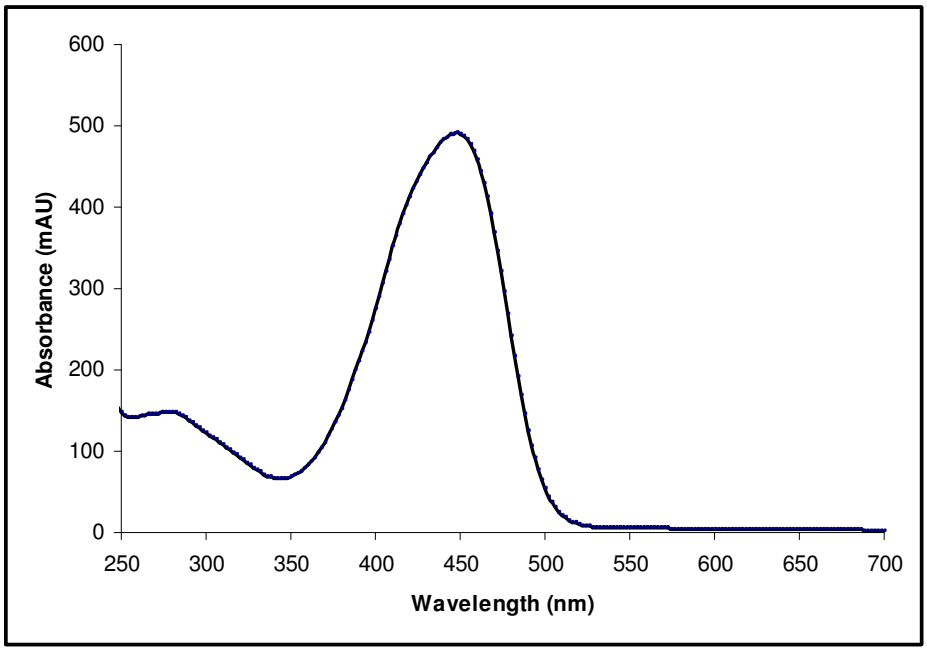

Figure S1. UV-VIS spectrum of bilirubin from *S. nicolai* (solvent system: 9:1 0.04 mM sodium acetate in methanol: 1% aqueous ammonium acetate).

Table S1. [1]HNMR values of bilirubin

Hydrogens	**Bilirubin from *S. nicolai***	**Multiplicity**	**Bilirubin Standard**
2-CH3	1.94	singlet	1.92
7-CH3	2.02 or 2.05	singlet	2.0 or 2.03
13-CH3	2.05 or 2.02	singlet	2.03 or 2.0
17-CH3	2.11	singlet	2.17
8 & 12-C*H2*CH2CO2H	2.46	triplet	2.43
8 & 12-CH2C*H2*CO2H	1.97	triplet (partly obscured)	1.94
10 CH2	4	singlet	3.99
18-CH=C*H2**	~5.30 (centroid)*	ABX system	~5.31 (centroid)*
18-C*H*=CH2*	~6.22 (centroid)*	ABX system	~6.21 (centroid)*
18-CH=CH2*	~6.6 (centroid)*	ABX system	~6.59 (centroid)*
3-CH=*CH2**	~5.63 (centroid)*	ABX system	~5.62 (centroid)*
3-CH=CH2*	~5.66 (centroid)*	AB*X* system	~5.66 (centroid)*
3-CH=CH2*	~6.84 (centroid)*	AB*X* system	~6.83 (centroid)*
5-H	6.1	singlet	6.1
15-H	6.1	singlet	6.1
21-H	10.1	broad singlet	10.04
22-H	10.49 or 10.52	broad singlet	10.45 or 10.48
23-H	10.52 or 10.49	broad singlet	10.48 or 10.45
24-H	9.97	broad singlet	9.92
COOH	12	broad singlet	11.9

*Coupling constants for isolated bilirubin and (bilirubin standard)
C-3: ABX unit: J_{A-B} = 2 Hz (2 Hz); J_{A-X} = 11.8 Hz (11.3 Hz); J_{B-X} = 17.4 Hz (17.1 Hz)
C-18: ABX unit J_{A-B} = 2.8 Hz (2.5 Hz); J_{A-X} = 11.3 Hz (11.5 Hz); J_{B-X} = 17.4 Hz (17.5 Hz)

Table S2. ^{13}CNMR values of bilirubin

Carbons	Bilirubin from *S. nicolai*	Published Bilirubin Values[10]
C-1 C=O	171.33	171.5
C-2	123.23	123.4
C-3	140.4	140.5
C-4	127.44	127.6
C-5 methine	98.9	99.2
C-6	122.03	122.1
C-7	119.56	119.6
C-8	123.42	123.4
C-9	130.79	130.6
C-10	23.6	23.7
C-11	131.45	131.4
C-12	124.1	124.0
C-13	119.77	119.8
C-14	122.26	122.3
C-15 methine	100.01	100.1
C-16	128.21	128.4
C-17	141.93	142.0
C-18	122.39	122.6
C-19 C=O	170.39	170.6
C-2'	9.43	9.5
C-3’	127.34	127.5
C-3’’	122.08	122.2
C-7'	9.11	9.2
C-8’(C-12’)	19.23	19.3
C-8’’(C-12’’)	34.02, 34.00	34.4
C-8’’’(C-12’’’)	173.95	174.1
C-13'	9.12	9.3
C-17'	9.23	9.4
C-18’ CH=CH2	127.06	127.2
C-18’’ CH=CH2	117.1	117.2